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## Two New Species and Three New Records of Ascomycetes in Korea

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### ABSTRACT

During a survey of plant-inhabiting fungi and water niches from Korea, noteworthy fungi were collected; among them, two new species, *Paracamarosporium noviaquum* sp. nov. and *Phyllosticta gwangjuensis* sp. nov., are described based on morphology and multi-gene phylogenies. *Paracamarosporium noviaquum* was characterized by its production of 1-celled and 2-celled conidia, forming conidiomata on only potato dextrose agar medium. *Phyllosticta gwangjuensis* was characterized by conidia hyaline, ovoid to ellipsoid shape, rounded at both ends, containing numerous guttulae or with a single large central guttule. Additional species were identified as *Cosmospora lavitskiae*, *Monochaetia cameliae*, and *Roussoella doimaesalongensis*, which are reported as new record species from Korea. Detailed descriptions and illustrations of these taxa are provided herein.

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### 1. Introduction

Fungi represent an integral part of the biomass of any natural environment. They act as predators, pathogens, and parasites to a myriad of other organisms and can be found living in symbiotic associations with plants, algae, and animals [1]. Ascomycota is the largest phylum in the kingdom Fungi and contains 92,724 species (Catalog of Life: <http://www.catalogueoflife.org/annual-checklist/2019/>; accessed September 20 2021). The phylum contains 3 subphyla and 20 classes [2]. Dothideomycetes is the largest and most ecologically diverse class of Ascomycota [3,4]. Currently, 36 orders, 167 families, 2102 genera, and 31,033 species are recognized (Catalog of Life: <http://www.catalogueoflife.org/annual-checklist/2019/>; accessed September 20 2021). Species of Dothideomycetes are found worldwide in terrestrial, freshwater, and marine habitats and are characterized mainly by bitunicate asci, usually with fissitunicate dehiscence [4–6]. Many members of Dothideomycetes are important plant pathogens [7–9], whereas some species are used in biotechnological applications [10,11]. Sordariomycetes is the second largest class of Ascomycota [3,12]. A total of 47 orders, 127 families, 1975 genera, and 23,187 species are currently recognized (Catalog of Life: <http://www.catalogueoflife.org/annual-checklist/2019/>; accessed September 20 2021). Sordariomycetes species are characterized by nonlichenized, perithecial ascomata, and

inoperculate unitunicate or nonfissitunicate asci [12,13]. Members in this class are found in both terrestrial and aquatic habitats [12,14,15]. Some species of Sordariomycetes are economically important as bio-control agents [16–18], and others produce a wide range of chemically diverse metabolites important in medicinal applications [19].

Hawksworth [20] estimated that there are approximately 1.5 million fungal species on Earth. However, an updated estimate of the number of fungal species is between 2.2 and 3.8 million [21], and aquatic fungal diversity has been estimated at 3000–4145 species [22,23]. Thus, a vast number of species remain to be discovered and described in aquatic habitats. Additionally, the importance of these organisms in secondary metabolite production has also been recognized [24,25].

In recent years, endophytic fungi have attracted increasing attention. Some researchers have shown that these organisms are an excellent source of biologically active compounds [26,27]; thus, exploring endophytic fungi that inhabit plants would provide opportunities for the discovery of new metabolites with potential bioactivity.

Despite the increasing number of investigations into the occurrence and distribution of fungi inhabiting plant and aquatic habitats, many new species of Dothideomycetes and Sordariomycetes remain undiscovered and uncharacterized in Korea [28–31].

The aim of this study was therefore to improve our understanding of the occurrence and distribution of species belonging to Sordariomycetes and Dothideomycetes from Korea and to describe two new species, *Paracamarosporium noviaquum* sp. nov., and *Phyllosticta gwangjuensis* sp. nov. This is also the first report of *Cosmospora lavitskiae*, *Monochaetia cameliae*, and *Roussoella doimaesalongensis* in Korea.

## 2. Materials and methods

### 2.1. Sample collection

Freshwater samples were collected from Wonhyo valley (35°09'01.2"N, 126°59'24.6"E) located in the Mudeung Mt., Gwangju, rainwater around Asiatic toad (*Bufo gargarizans*) in a garden located in Kunryang-ri (36°26'16.2"N, 126°46'04.6"E), Cheongyang-eup, Cheongyang, Chungnam Province, and pondwater at Hanbat arboretum (36°21'56.4"N, 127°23'16.3"E) located in Daejeon. Seawater samples were collected at Kkotji Beach (36°29'49.1"N, 126°20'06.0"E), Anmyeondo Island, Chungnam Province. The samples were collected in sterile plastic bags or sterile 50-mL Falcon tubes and transferred to the laboratory.

Fresh and healthy leaves of *Torreya nucifera* were collected from the arboretum of Chonnam National University (35°10'20.3"N, 126°53'56.4"E) located in Gwangju, Korea. Materials were kept in zip-lock bags while they were transferred to the laboratory. Fungal isolation was carried out within 24 h of collection.

### 2.2. Isolation of fungal strains

To isolate fungi from leaves samples, the plant leaves were washed thoroughly in running tap water. Leaf tissue pieces were cut into small fragments (0.5 × 0.5 cm) using a sterilized blade. The cut segments were surface sterilized by immersing in 70% (v/v) ethanol for 60 s, and then placed in 2% sodium hypochlorite for 60 s, and a final rinsing

using sterile distilled water three times. Samples were dried on sterilized filter paper. Small fragments were placed on potato dextrose agar (PDA; 39 g PDA; Becton, Dickinson and Co., Sparks, MD, USA, and 1 L deionized water) medium with antibiotic (100 mg/L streptomycin sulfate) and incubated at 25 °C until fungal growth emerged from the plant segments. Hyphal tips were transferred to new PDA plate.

Fungal isolation from water samples were conducted following our previous methods [32]. The cultures were maintained in PDA slant tubes and in 20% glycerol at –80 °C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea. Type specimens were deposited at Chonnam National University Herbarium, Gwangju, Korea as inactive dried cultures. Information on all isolates used in this study is shown in Table 1.

### 2.3. Morphological studies

The strains were inoculated onto PDA, malt extract agar (MEA; 20 g of malt extract and 20 g of agar in 1 L of deionized water), and oatmeal agar (OA; 30 g of oatmeal and 20 g of agar in 1 L of deionized water). The plates were incubated at 25 °C in the dark for 7 days. Colony characters were recorded after 7 days. For morphological observation, fragments were removed from cultures and placed on microscope slides with lactic acid (60%) and observed under light a microscopy (Olympus BX53, Tokyo, Japan). Scanning electron microscopy was performed as described previously [32].

### 2.4. DNA extraction, polymerase chain reaction, purification, and sequencing

Genomic DNA was extracted from fresh fungal mycelia that were grown on cellophane at 25 °C for 5 days using the Solg<sup>TM</sup> Genomic DNA Preparation Kit (Solgent Co. Ltd., Daejeon, Korea). The ITS and LSU regions were amplified using the primer pairs V9G and ITS4 [33], and LROR and LR5 [34,35],

**Table 1.** Source information and GenBank accession numbers of the studied strains.

Species	Strain number	Source	GenBank accession no.
			ITS/LSU/RPB2/ACT/TEF1
<i>Cosmospora lavitskiae</i>	CNUFC TKB240= NNIBRFG31607	Rainwater	OK285197/-/OK431604
<i>Monochaetia cameliae</i>	CNUFC KOW41= QWJQFGC000000439	Seawater	OK285198
<i>Paracamarosporium noviaquum</i>	CNUFC MSW24-4= CNFG_3191	Freshwater	OK285193/OK315641
<i>Pa. noviaquum</i>	CNUFC MSW24-4-1	Freshwater	OK285194/OK315642
<i>Phyllosticta gwangjuensis</i>	CNUFC NJ1-12= IMYKFGC000000079	Leaf of <i>Torreya nucifera</i>	OK285195/-/OM001471/OM038511
<i>P. gwangjuensis</i>	CNUFC NJ1-12-1	Leaf of <i>Torreya nucifera</i>	OK285196/-/OM001472/OM038512
<i>Roussoella doimaesalongensis</i>	CNUFC WT16= NNIBRFG31619	Pondwater	OK285199/OK315643

respectively. Second largest subunit of RNA polymerase (*RPB2*) was amplified using the primer pairs RPB2-5F and RPB2-7cR [36]. The actin (*ACT*) gene was amplified using the primer pairs ACT-512F and ACT-783R [37]. To amplify the elongation factor (*TEF1*) gene region, the primer pairs EF1-728F and EF1-986R [37] were used. Polymerase chain reaction (PCR) amplification of the ITS, LSU, *RPB2*, *ACT*, and *TEF1* gene regions was performed according to the conditions described by Liu et al. [36], Carbone and Kohn [37], and Nguyen et al. [38]. The quality of PCR products was assessed using 1% agarose gel electrophoresis. The PCR products were purified with the Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, Korea), and sequencing was performed using the same PCR primers with the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at Macrogen (Daejeon, Korea).

## 2.5. Phylogenetic analyses

Sequences for the selected strains were retrieved from GenBank, and aligned using MAFFT (<http://mafft.cbrc.jp/alignment/server>) with the L-INS-I algorithm. Aligned sequences were automatically trimmed using trimAl [39] with the gappyout method. Phylogenetic reconstructions by the maximum likelihood (ML) method were conducted using RAXML-HPC2 on XSEDE on the online CIPRES Portal (<https://www.phylo.org/portal2>) with 1000 bootstrap replicates and the GTRGAMMA model of nucleotide substitution. A Bayesian inference (BI) analysis was performed with MrBayes 3.2.2 [40] using a Markov Chain Monte Carlo algorithm. BI analysis was conducted with  $1 \times 10^6$  generations, a burning value of 25%. The trees were visualized using FigTree v. 1.3.1 [41]. Values less than 0.95 BI posterior probability (BPP) and 70% ML bootstrap (MLBS) are not shown. The newly obtained sequences were deposited in the GenBank database with the accession numbers shown in Table 1.

## 3. Results

### 3.1. Molecular phylogeny

In the phylogenetic analysis based on the combined ITS and LSU sequence data, *Paracamarosporium noviaquum* forms a distinct lineage from *Pa. fungicola* with 100% MLBS and 1 BPP support values (Figure 1). Multi-gene phylogenetic analysis of the combined ITS, *ACT*, and *TEF1* dataset indicated that *Phyllosticta gwangjuensis* is related to

*P. aucubae-japonicae* with 91% MLBS and 1 BPP support values (Figure 2). Phylogenetic analysis of the combined ITS and *RPB2* dataset indicated that our strain CNUFC TKB240 clustered together with *Cosmospora lavitskiae* CBS 530.68 (ex-type) with 100% MLBS and 1 BPP support values (Figure 3). Phylogenetic analysis of ITS sequence showed that our strain CNUFC KOW41 clustered among *Monochaetia camelliae* strains (ICMP 10669, PSH2000I-151, and ATCC 60625) with 100% MLBS and 1 BPP support values (Figure 4). The phylogenetic analysis of the combined ITS and LSU sequence dataset showed that CNUFC WT16 clusters with the ex-type strain of *Roussoella doimaesalongensis* MFLUCC 14-0585 with 99% MLBS and 0.99 BPP support values (Figure 5).

### 3.2. Taxonomy

***Paracamarosporium noviaquum*** Hyang B. Lee, H.J. Lim and T.T.T. Nguyen sp. nov.

Index Fungorum number: 557725 (Figure 6).

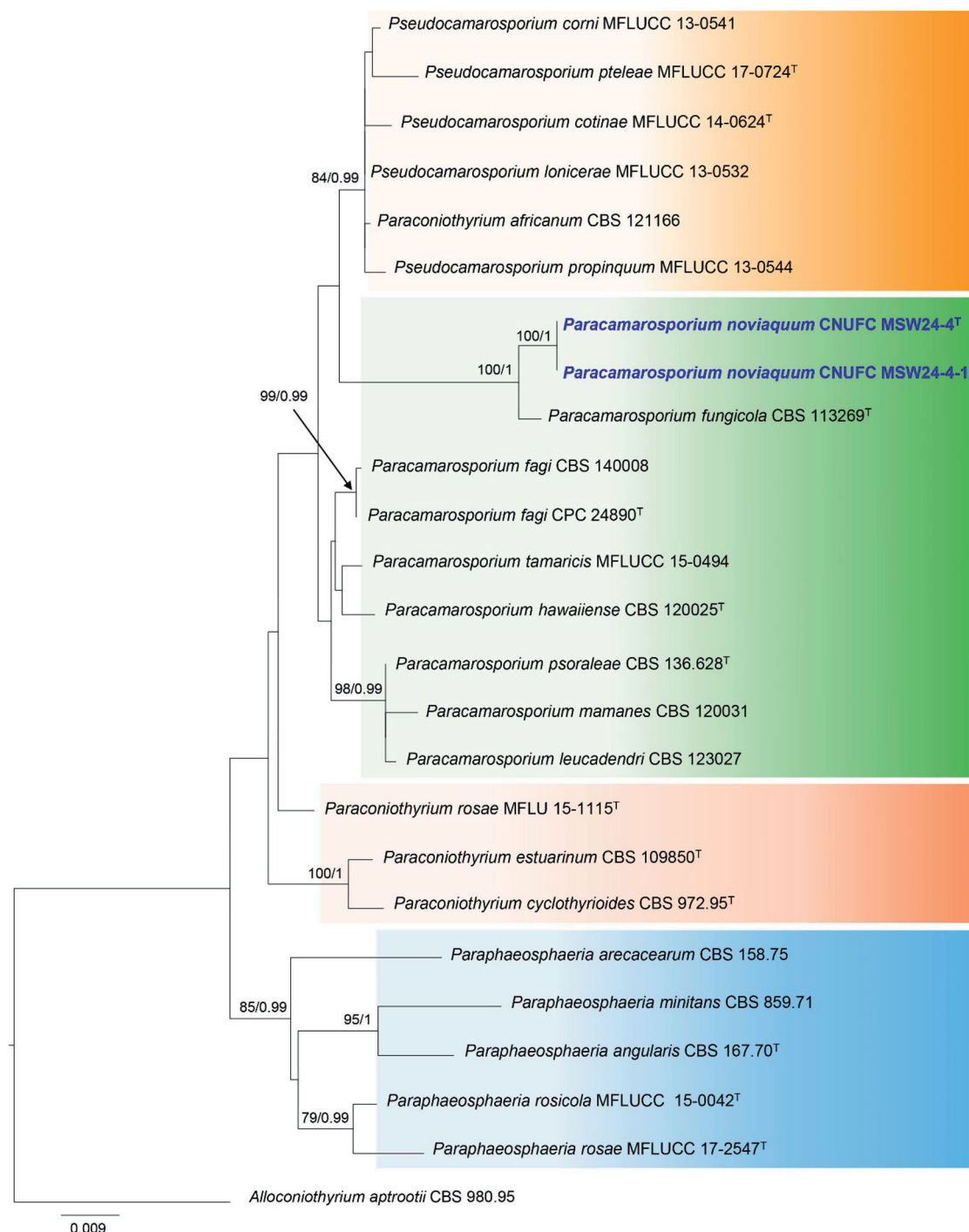
Etymology: Named after the source of freshwater, where this fungus was collected.

Description: Colonies initially white to smoke gray and then slightly brown to bluish green, floccose, sulcate; reverse dark brown in the center. Conidiomata observed after 1 month, superficial or immersed, dark brown to black, eustromatic, composed of a tissue of textura angularis-globulosa, sometimes with central papillate ostioles,  $66\text{--}480 \times 57\text{--}348 \mu\text{m}$ . Conidiogenous cells formed from inner cells all over the conidiomatal wall, discrete, phialidic, hyaline, ampulliform,  $5\text{--}8\text{--}(10) \times 3.5\text{--}5 \mu\text{m}$ . 1-celled conidia irregular, colorless, ovoid to ellipsoid shape, broadly rounded at both ends,  $(3.5\text{--})4\text{--}5.5 \times 2.5\text{--}3.5 \mu\text{m}$ . 2-celled conidia produced after nearly 3 months, brown, cylindrical, reniform,  $7.5\text{--}10 \times 3.0\text{--}4.5 \mu\text{m}$ .

Culture characteristics: CNUFC MSW24-4 showed varying growth rates at 25 °C on PDA, MEA, and OA, reaching 23, 32.5, and 31.5 mm after 21 days, respectively. The optimal growth temperature was around 25 °C, with no growth at 35 °C.

Material examined: REPUBLIC OF KOREA, South Jeolla Province, Gwangju, Wonhyo valley located in the Mudeung Mt. ( $35^{\circ}09'01.2''\text{N}$ ,  $126^{\circ}59'24.6''\text{E}$ ), from freshwater, January 2017, H.B. Lee, CNUFC HT17024 (holotype), ex-type living culture (CNUFC MSW24-4).

Notes: The multigene analysis (Figure 1) indicates that *Paracamarosporium noviaquum* is closely related to *Pa. fungicola* (Verkley & Wicklow) Wijayaw & Hyde. These two species have similar morphological characteristics of conidiomata,



**Figure 1.** Maximum likelihood phylogenetic tree inferred from combined dataset of ITS and LSU sequence data for *Paracamarosporium noviaquum* CNUFC MSW24-4, *Pa. noviaquum* CNUFC MSW24-4-1, and related species. Bootstrap support values for maximum likelihood (MLBS) higher than 70% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above or below branches. *Alloconiothyrium aptrootii* CBS 980.95 was used as the outgroup. The newly generated sequences are indicated in bold blue. T = ex-type.

conidiogenous cells, and conidia. However, *Pa. noviaquum* differs from *Pa. fungicola* by producing conidiomata on PDA medium only and has smaller 1-celled conidia and larger 2-celled conidia. Moreover, *Pa. noviaquum* grew slower than *Pa. fungicola* on all media after 21 days (MEA 35–38 mm; PDA: 60–63 mm; OA: 65 mm) [42].

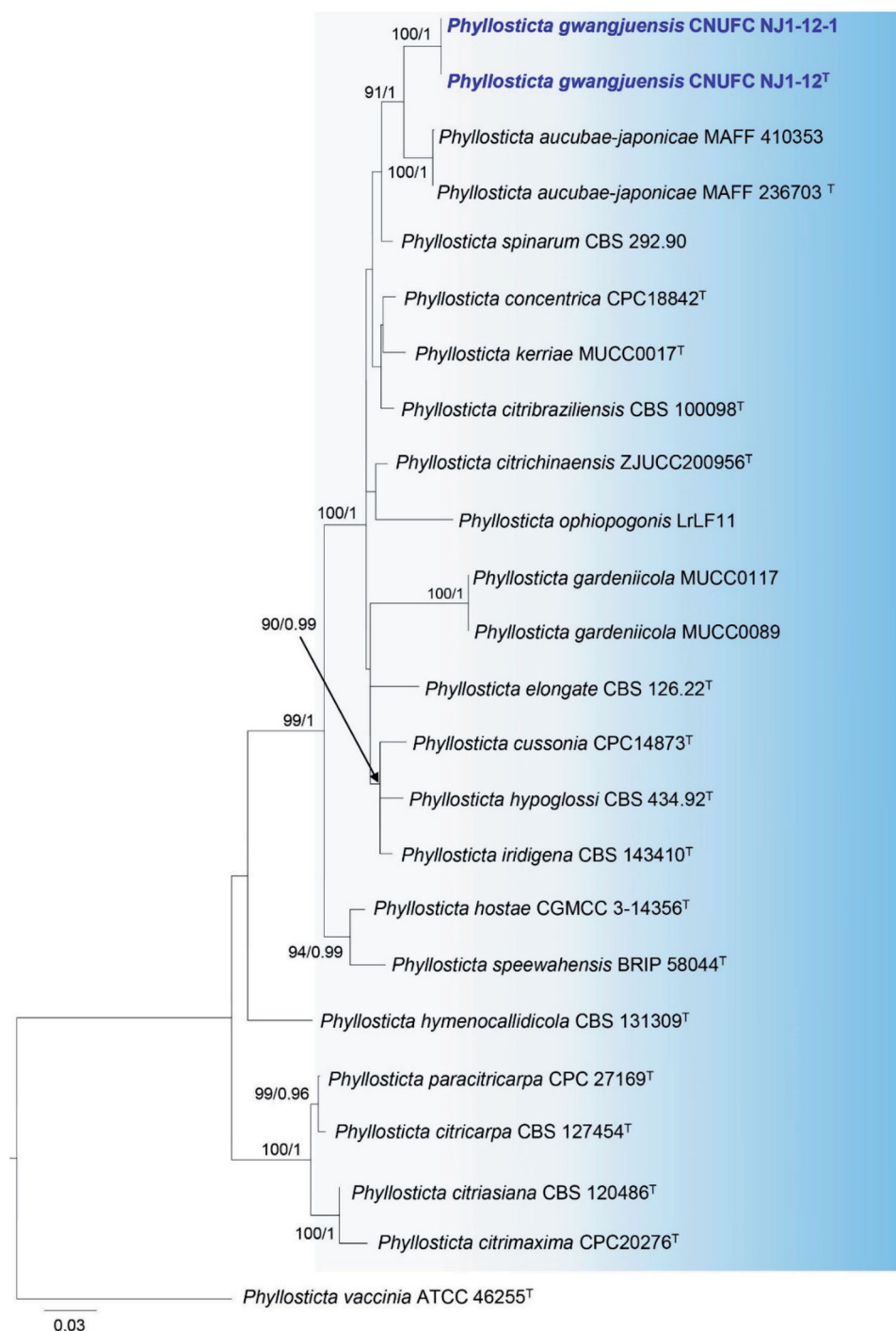
***Phyllosticta gwangjuensis*** Hyang B. Lee, H.J. Lim and T.T.T. Nguyen sp. nov.

Index Fungorum number: 557003 (Figure 7).

**Etymology:** Refers to the location from where the strain was isolated.

**Description:** Colonies black to dark gray, colorless, flat with irregular margin; reverse black to dark



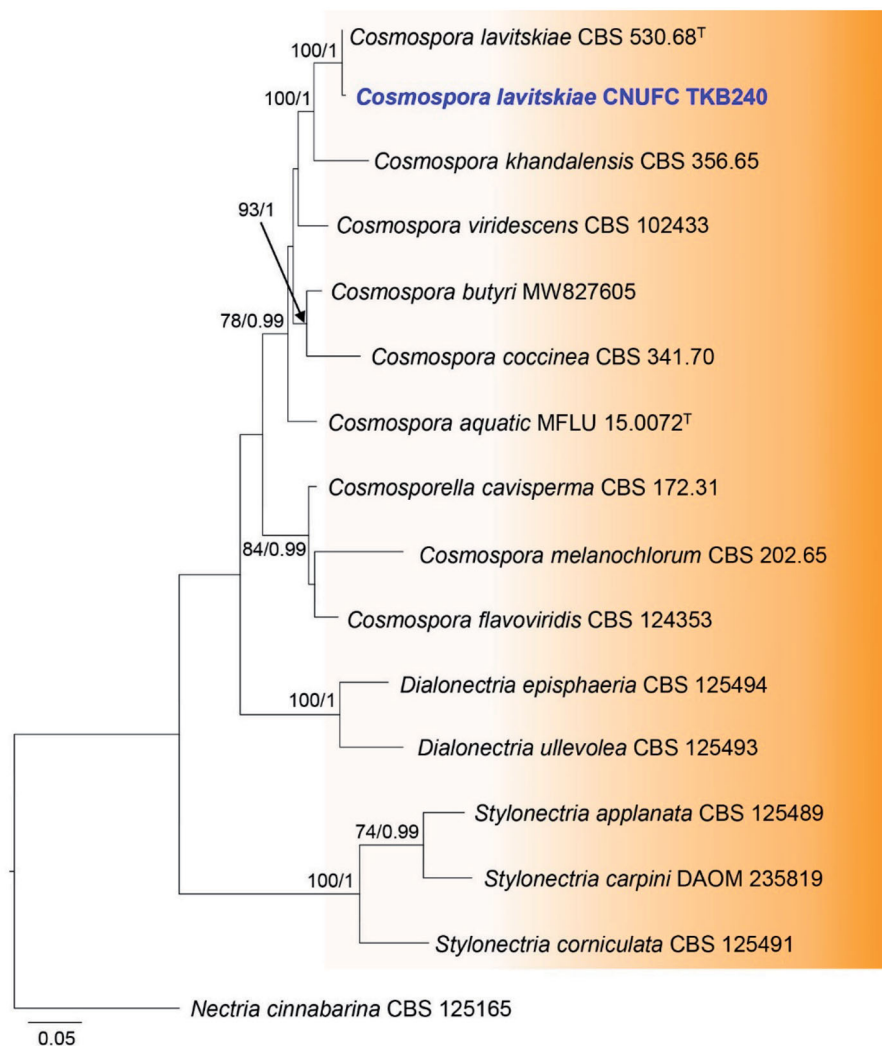


**Figure 2.** Maximum likelihood phylogenetic tree inferred from combined ITS, *TEF1*, and *ACT* gene sequence data for *Phyllosticta gwangjuensis* CNUFC NJ1-12, *P. gwangjuensis* CNUFC NJ1-12-1, and related species. Bootstrap support values for maximum likelihood (MLBS) higher than 70% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above or below branches. *Phyllosticta vaccinia* ATCC 46255 was used as the outgroup. The newly generated sequences are indicated in bold blue. T = ex-type.

gray. Conidiomata pycnidial, dark brown to black, usually aggregated, sometimes decentralized, subglobose, irregular, and exuding opaque conidial masses. Conidiogenous cells cylindrical, subcylindrical, ampulliform, hyaline,  $(7.5\text{--})8.5\text{--}22.5\text{--}(23.5) \times (3\text{--})3.5\text{--}5.5\text{--}(6) \mu\text{m}$ . Conidia hyaline, ovoid to ellipsoid shape, rounded at both ends, containing

numerous guttulae or with a single large central guttule,  $(8.5\text{--})10\text{--}13.5 \times 7\text{--}9\text{--}(9.5) \mu\text{m}$ . Appendages hyaline, straight, flexible, deciduous,  $(4.5\text{--})5\text{--}29\text{--}(43.5) \mu\text{m}$  long.

Culture characteristics: CNUFC NJ1-12 showed different growth at 25 °C on PDA, MEA, and OA, reaching 34 mm, 28 mm and 27.5 mm at 14 days,



**Figure 3.** Maximum likelihood phylogenetic tree inferred from combined ITS and *RPB2* gene sequence data for *Cosmospora lavitskiae* CNUFC TKB240, and related species. Bootstrap support values for maximum likelihood (MLBS) higher than 70% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above or below branches. *Nectria cinnabarina* CBS 125165 was used as the outgroup. The newly generated sequences are indicated in bold blue. T = ex-type.

respectively. The optimal growth temperature was observed around 25 °C, with no growth at 35 °C.

Material examined: REPUBLIC OF KOREA, South Jeolla Province, Gwangju, Chonnam National University arboretum (35°10'20.3"N, 126°53'56.4"E), from leaf of *Torreya nucifera*, November 2018, H.B. Lee, holotype (CNUFC HT18112), ex-type living culture (CNUFC NJ1-12).

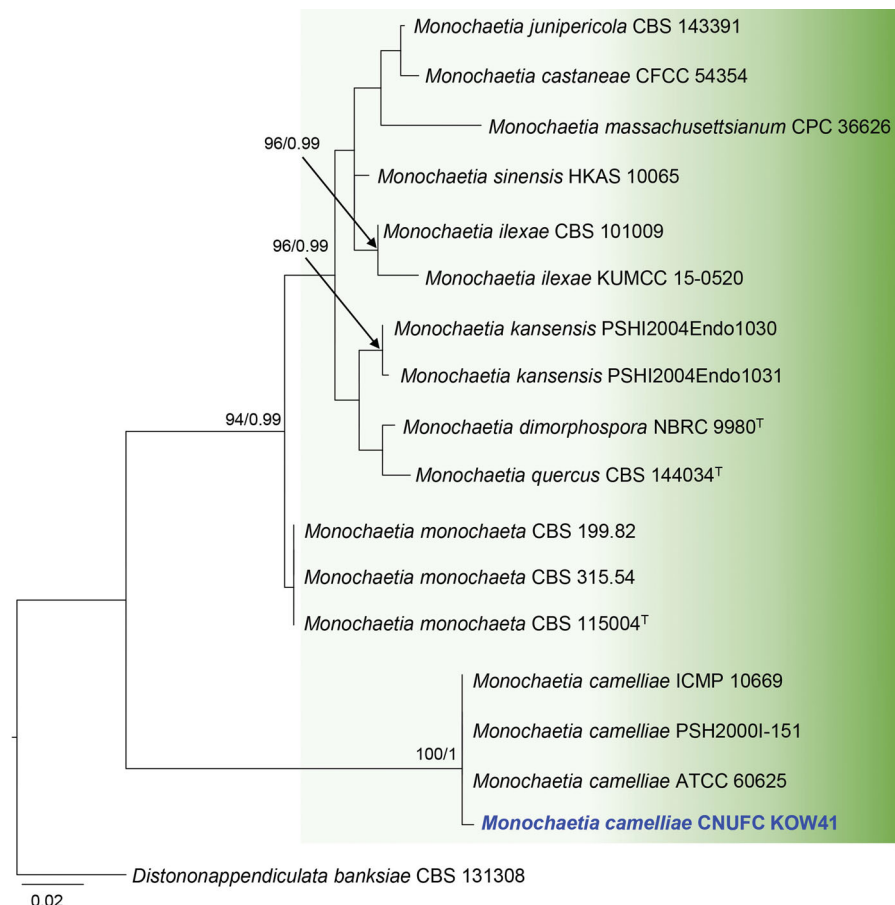
Notes: The multigene analysis (Figure 2) indicates that *Phyllosticta gwangjuensis* is closely related to *P. aucubae-japonicae* [43]. *Phyllosticta gwangjuensis* has similar morphology to *P. aucubae-japonicae*, but this species is distinguished by producing longer conidiogenous cells and appendages than those by *P. aucubae-japonicae*, and conidia contain numerous guttulae or with a single large central guttule.

***Cosmospora lavitskiae*** (Zhdanova) Gräfenhan & Seifert 2011 (Figure 8).

≡ *Gliomastix lavitskiae* Zhdanova, Mikrobiol. Zh. 28: 37. 1966.

Description: Colonies grew slowly on PDA, texture velvety, slightly raised at center, yellow, irregular margin, soluble pigment, and reached 15 mm in diameter after 7 days at 25 °C; reverse umber. Conidiophores unbranched, with a terminal whorl of one to three phialides. Phialides monophialidic, cylindrical, 40–62(–70.5) μm long, 2–3 μm wide at base, 1.5–2 μm wide at apex. Conidia ellipsoidal, smooth, hyaline, 3.5–5.5 × 2.0–2.5 μm.

Notes: The observed characteristics were mostly similar to those previously described for *Cosmospora lavitskiae* [44], although there was a difference in the size of phialides and conidia. The size of phialides and conidia described by Zhdanova [44] were 31–50 μm long and 3.5–6 × 1–3 μm, respectively, whereas our isolates were 40–62(–70.5) and 3.5–5.5 × 2.0–2.5 μm, respectively. *Cosmospora lavitskiae* are isolated from soil rhizosphere of *Zea mays* and found on xylariaceous fungus [44–46]. The strain in this study was isolated from fresh-water samples.



**Figure 4.** Maximum likelihood phylogenetic tree inferred from ITS sequence data for *Monochaetia camelliae* CNUFC KOW41, and related species. Bootstrap support values for maximum likelihood (MLBS) higher than 70% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above or below branches. *Distononappendiculata banksiae* CBS 131308 was used as the outgroup. The newly generated sequences are indicated in bold blue. T = ex-type.

Specimen examined: REPUBLIC OF KOREA, Chungnam Province, Cheongyang, Cheongyang-eup, Kunryang-ri (36°26'16.2"N 126°46'04.6"E), from rainwater around *Bufo gargarizans*, June 1 2020, J.S. Kim (culture CNUFC TKB240).

*Monochaetia camelliae* Miles, Mycologia 18: 167 (1926) (Figure 9).

Description: Colonies grew fast on PDA, white in color, and reached 70 mm in diameter after 7 days at 25 °C; reverse yellowish white in color. Conidiogenous cells subcylindrical, hyaline, smooth. Conidia fusoid, mostly four-septate, occasionally three-septate, usually not constricted at the septa, measured (11–)16.5–20 × 4–6 µm, bearing appendages. Appendage tubular, attenuated, and single; apical appendages single, straight, or oblique, variable in size, 9–16 µm long.

Notes: The morphological descriptions of CNUFC KOW41 matched well with the previously described species of *Monochaetia camelliae* [47]. However, our observations showed that the size of conidia of type species described by Miles [47] was narrower than that of our isolate CNUFC KOW41. *Monochaetia camelliae* were described as four-septate [47], whereas they were three to four septate in our strain. *Monochaetia camelliae* is commonly

isolated from leaves of *Camellia japonica*, *C. hongkongensis*, and *C. pitardii* [47,48]. The Korean isolate CNUFC KOW41 was found in seawater samples.

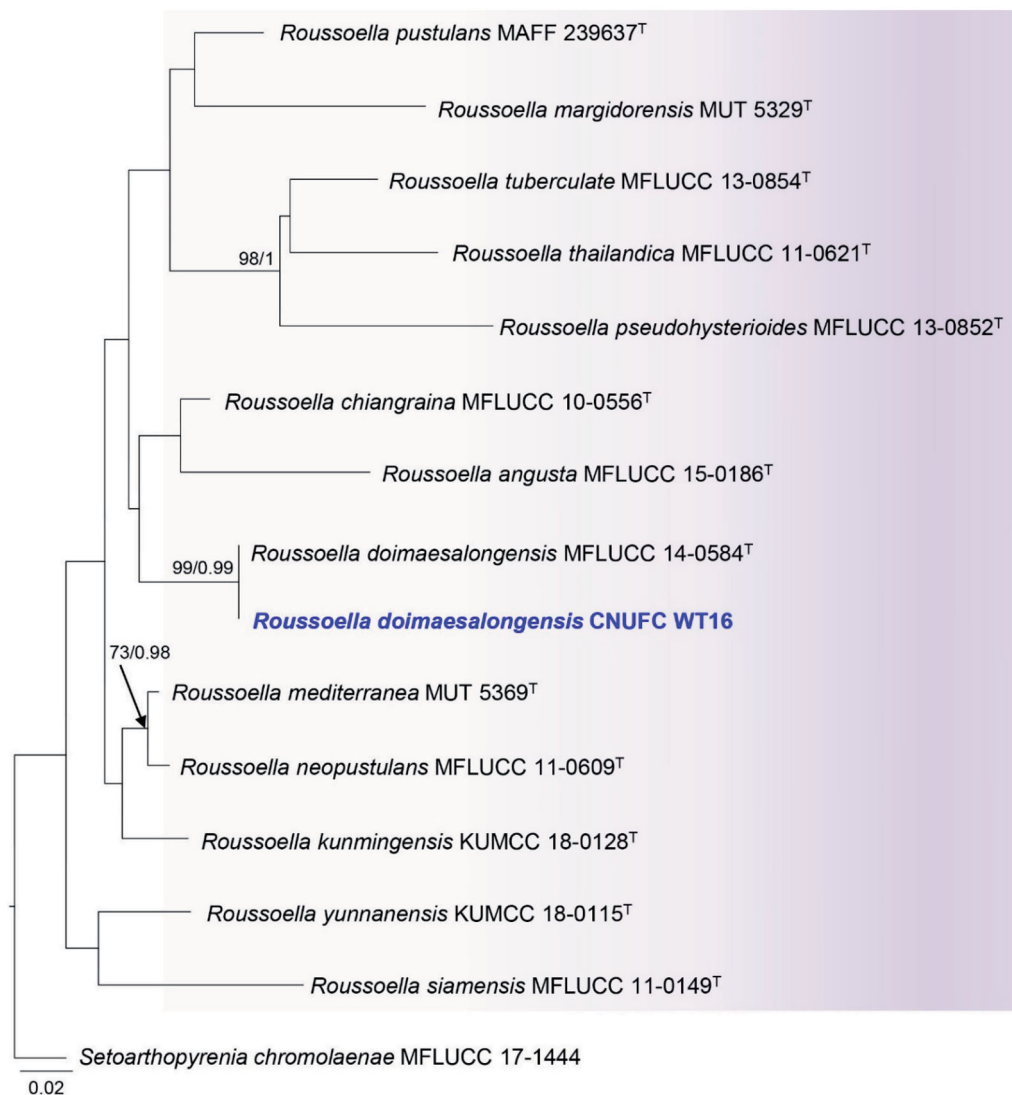
Specimen examined: REPUBLIC OF KOREA, Chungnam Province, Anmyeondo Island, Kkotji Beach (36°29'49.1"N, 126°20'06.0"E), from seawater, May 2019, H.B. Lee (culture CNUFC KOW41).

*Roussoella doimaesalongensis* Thambug. & K.D. Hyde, Mycosphere 8 (4): 782 (2017) (Figure 10).

Description: Colonies grew slowly on PDA, brownish green, surface velvety, white to near the margin, and reached 32 mm in diameter after 7 days at 25 °C; reverse grayish black with lighter edges. Hyphae 1.5–3 µm wide, septate, brown in color. Chlamydospores 2.5–8.5 µm, globose to subglobose, intercalary, or terminal.

Notes: Based on the current phylogenetic analysis, our strains were grouped within the same clade as *Rousoella doimaesalongensis* (ex-type strain) (Figure 5). *Rousoella doimaesalongensis* is found on decaying bamboo culms from Thailand [49]. The Korean isolate CNUFC WT16 was isolated from freshwater samples. All attempts to induce sporulation failed.

Specimen examined: REPUBLIC OF KOREA,  
Daejeon, Hanbat arboretum (36°21'56.4"N,



**Figure 5.** Maximum likelihood phylogenetic tree inferred from combined ITS and LSU sequence data for *Roussoella doimaesalongensis* CNUFC WT16, and related species. Bootstrap support values for maximum likelihood (MLBS) higher than 70% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above or below branches. *Setoarthopyrenia chromolaenae* MFLUCC 17-1444 was used as the outgroup. The newly generated sequences are indicated in bold blue. T = ex-type.

127°23'16.3"E), from pondwater, February 2020, H.B. Lee (culture CNUFC WT16).

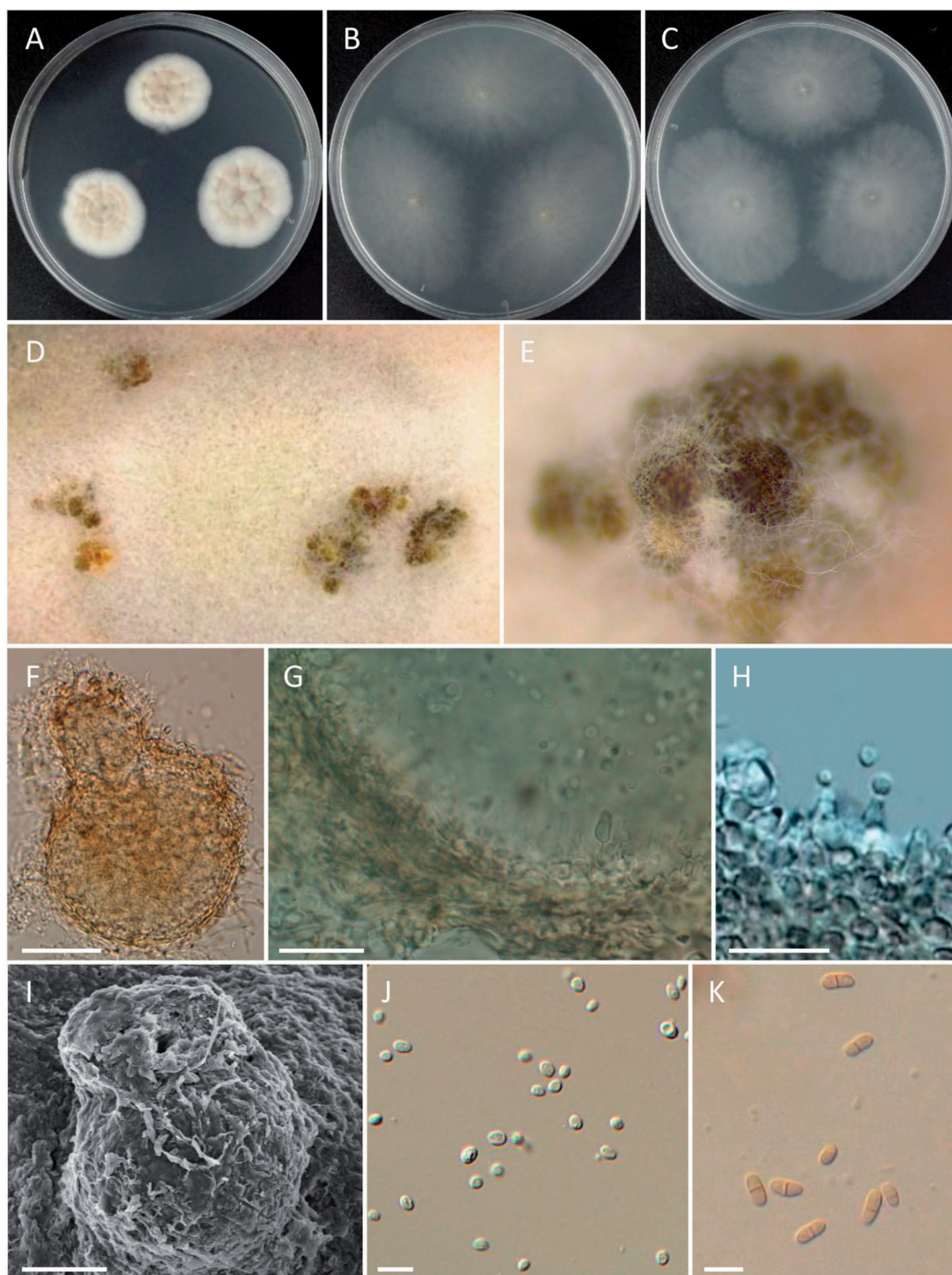
#### 4. Discussion

In this study, two new species and three new records in Dothideomycetes and Sordariomycetes are described and illustrated. Our phylogenetic analysis based on ITS and LSU rDNA dataset indicated *Paracamarosporium noviaquum* is closely related to *Pa. fungicola* (Figure 1), which also has both 1-celled and 2-celled conidia. However, *Pa. noviaquum* has smaller 1-celled conidia and larger 2-celled conidia, and producing conidiomata on PDA medium only. Growth on MEA, PDA, and OA at 25 °C can be easily used to distinguish between *Pa. noviaquum* and *Pa. fungicola* [42]. Wijayawardene et al. [50] introduced genus *Paracamarosporium* to accommodate *Camarosporium psoraleae*, which is

characterized by paraphyses and microconidia. Crous et al. [51] transferred *Microdiplodia hawaiiensis* and *Camarosporium leucadendri* to *Paracamarosporium* as *Pa. hawaiiense* and *Pa. leucadendri*, respectively. In 2016, Wijayawardene et al. [52] transferred *Paraconiothyrium fungicola* to genus *Paracamarosporium* as a new combination. Recently, two species, namely, *Pa. tamaricis* and *Pa. mamanan*, were added to this genus [53,54]. Currently, this genus consists of seven species (Index Fungorum 2021; [www.indexfungorum.org](http://www.indexfungorum.org)). Several potentially novel secondary metabolites have been detected in the species *Pa. leucadendri* [55]. Thus, further studies are needed to isolate and identify secondary metabolites from new species, *Pa. noviaquum*.

*Phyllosticta gwangjuensis* is phylogenetically related to *P. aucubae-japonicae* (Figure 2). However, *P. gwangjuensis* produces longer conidiogenous cells



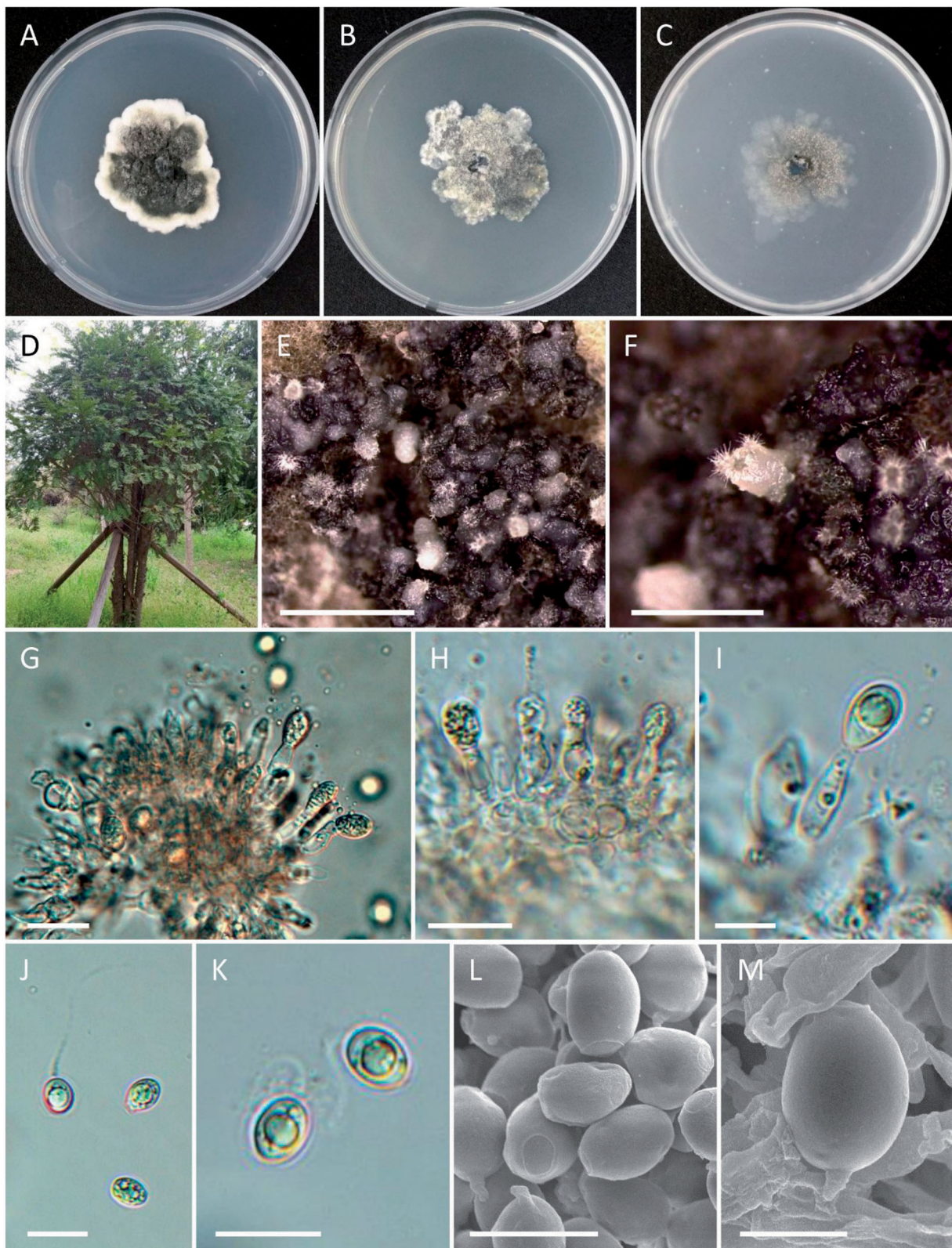


**Figure 6.** Morphology of *Paracamarosporium noviaquum*. (A) Colonies on PDA; (B) Colonies on MEA; (C) Colonies on OA; (D, E) Conidiomata on PDA; (F) Pycnidium; (G, H) Conidiogenous cells on pycnidial wall; (I) Ostiole of pycnidium; (J) 1-celled conidia; (K) 2-celled conidia; (D, E) Observed using stereo-microscope; (F–H, J, K) LM; (I) SEM (scale bars: F = 100  $\mu$ m; G, H = 20  $\mu$ m, I = 40  $\mu$ m; J, K = 10  $\mu$ m).

and appendages. Especially, *P. gwangjuensis* has a large guttule in the conidia. The genus *Phyllosticta* was first described by Persoon [56] and typified by *P. convallariae* Pers. The main morphological

characteristics of the genus are pycnidia and conidia with a single apical appendage [57]. Species of *Phyllosticta* are well known as plant pathogens, forming black spots on the leaf, stem, or fruit, and





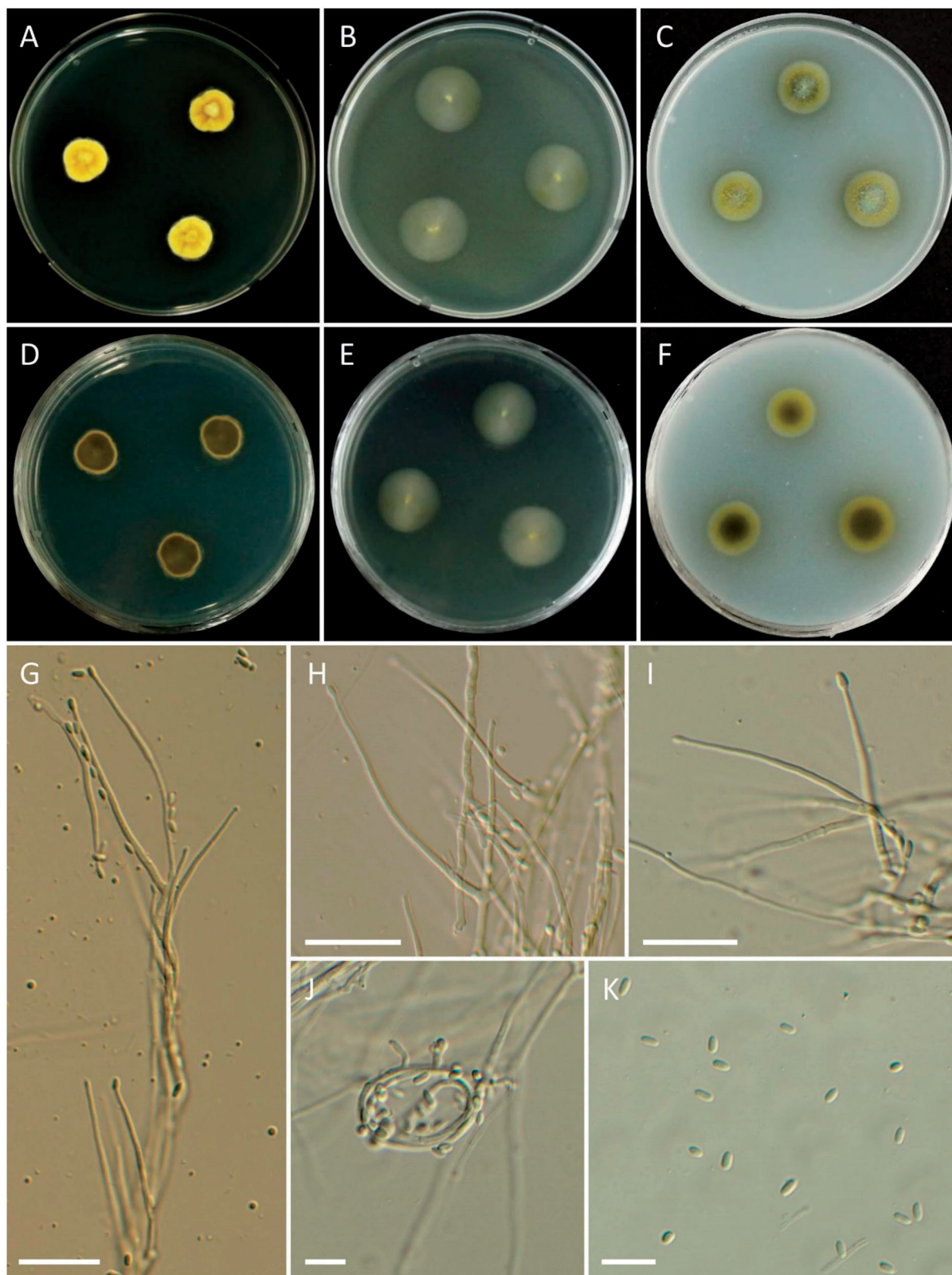
**Figure 7.** Morphology of *Phyllosticta gwangjuensis*. (A) Colonies on PDA; (B) Colonies on MEA; (C) Colonies on OA; (D) *Torreya nucifera* as host plant; (E, F) Conidiomata; (G–I) Conidiogenous cells and conidia; (J–M) Conidia; (L, M) SEM (scale bars: E = 2 mm; F = 1 mm; G, H = 20 µm; I–L = 10 µm; M = 5 µm).

have been found as endophytes or saprobes [58–61]. Additionally, several species of this genus have been associated with *Torreya nucifera* [62]. It seems that *Phyllosticta* species are widely distributed and appear to have different hosts. These results suggest that there may be a large number of undescribed

species, both endophytes and pathogens, from *Torreya nucifera*.

The strain CNUFC TKB240 clustered with *Cosmospora lavitskiae* CBS 530.68 (type species), with well-supported branches, in full agreement with a previous study conducted by Gräfenhan et al.



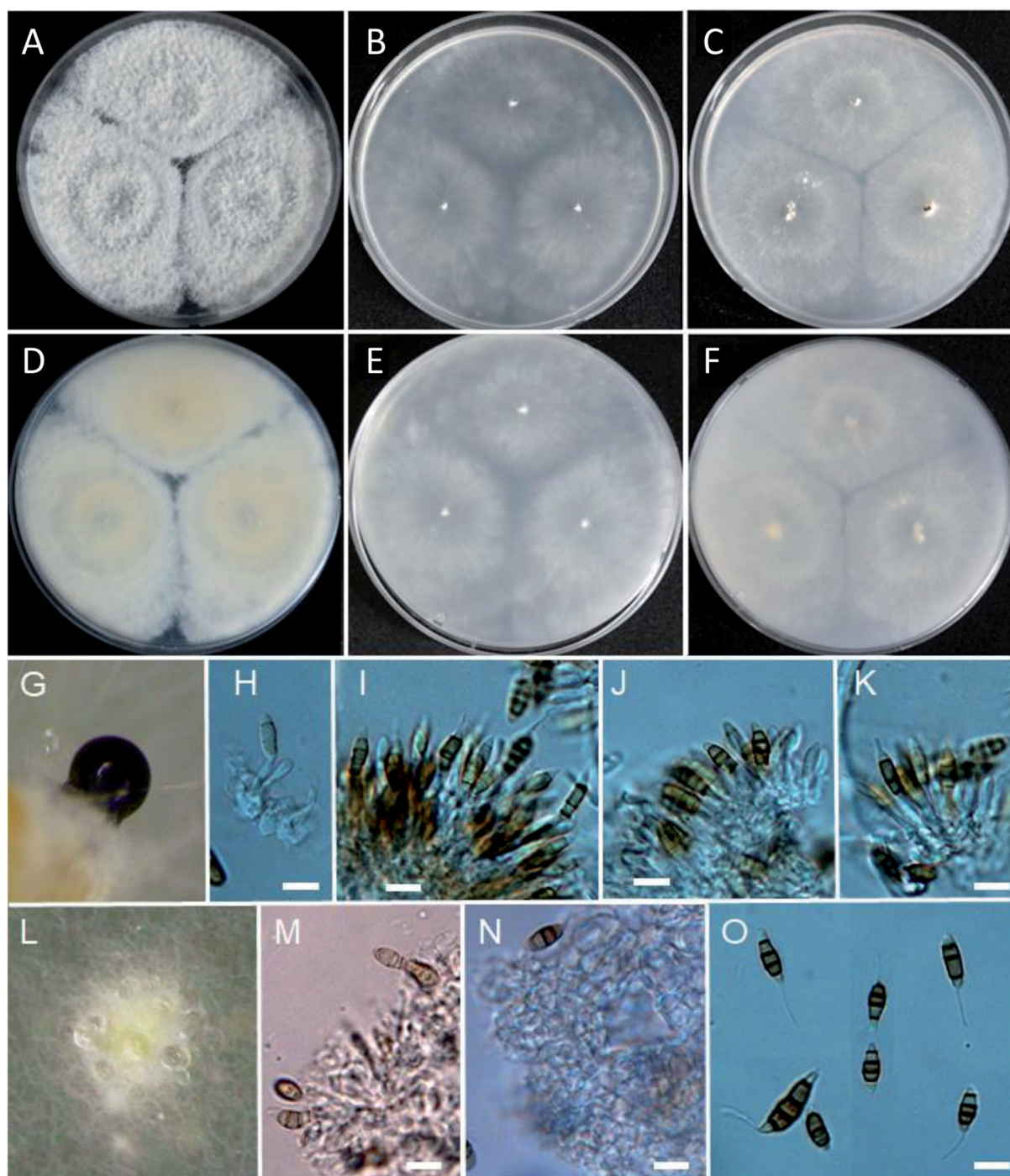


**Figure 8.** Morphology of *Cosmospora lavitskiae*. (A, D) Colonies on PDA; (B, E) Colonies on MEA; (C, F) Colonies on OA ((A–C) obverse view; (D–F) reverse view); (G–I) Phialides on conidiophores; (J) Coiled hyphae; (K) Conidia (scale bars: G–I = 20  $\mu$ m; J, K = 10  $\mu$ m).

[45]. *Cosmospora* is the genus in the family Nectriaceae (Hypocreales, Sordariomycetes) and was introduced by Rabenhorst [63] with the type species *C. coccinea* [64]. Currently, there are 49 accepted species in this genus (Index Fungorum 2021). *Cosmospora lavitskiae* was first isolated from soil

rhizosphere of *Zea mays* [45]. *Cosmospora lavitskiae* has been recorded for the first time in Korea, and this is also the first report from a rainwater.

Phylogenetic analysis based on ITS sequence indicated the CNUFC KOW41 strain was clustered with other strains of *M. cameliae* (Figure 4). The results



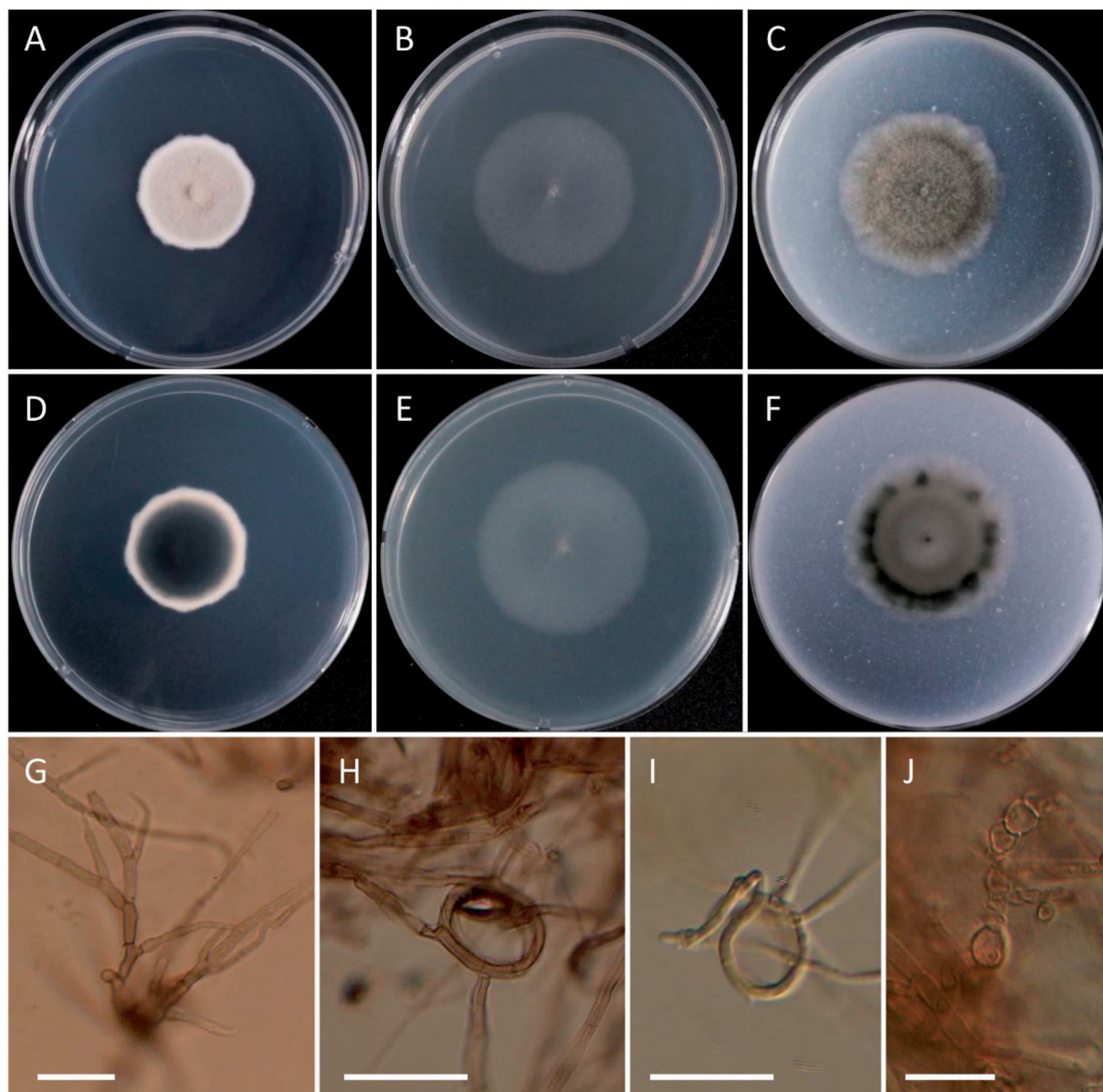
**Figure 9.** Morphology of *Monochaetia camelliae*. (A, D) Colonies on PDA; (B, E) Colonies on MEA; (C, F) Colonies on OA ((A–C) obverse view; (D–F) reverse view); (G) Conidiomata; (H–K, M) Conidiogenous cells and conidia; (L) Exudate on OA; (N) Conidiomatal wall; (O) Conidia (scale bars: H–K, M–O = 10 µm).

of our molecular analysis were consistent with the phylogeny presented by Liu et al. [48]. The genus *Monochaetia* was introduced by Allescher in 1902 [65]. *Monochaetia* belongs to the family Sporocadaceae (Amphisphaeriales, Sordariomycetes), characterized by three- to five-septate hyaline to brown conidia with single apical and basal appendages [66]. Species of *Monochaetia* are often found to be plant pathogens that cause post-harvest losses. Some species, such as *M. karstenii* produces different metabolites such as cyclohexenone derivatives, cinnamic acid, isooxazoline 3-phenyl-

benzodiazepine, 2-propenoic acid, 3-phenyl-(E)-dodecene, and 3-undecen-1-yne (E) with antimicrobial and antioxidant activity [67]. *Monochaetia* sp. Tbp-2 can produce paclitaxel [68]. Therefore, it is possible that a vast number of novel metabolites remain to be discovered from this genus.

Phylogenetic analysis based on ITS and LSU sequences showed that CNUFC WT16 clustered within the same clade with *Rousoella doimaesalonensis* MFLUCC 14-0584 (Figure 5). *Rousoella* is a genus in the family Rousoellaceae (Pleosporales, Dothideomycetes) and typified by *R. nitidula*. This





**Figure 10.** Morphology of *Roussioella doimaesalongensis*. (A, D) Colonies on PDA; (B, E) Colonies on MEA; (C, F) Colonies on OA ((A–C) obverse view; (D–F) reverse view). (G–I) Hyphae and coiled hyphae; (J) Chlamydospores (scale bars: G–I = 20  $\mu$ m; J = 10  $\mu$ m).

genus was introduced by Saccardo and Paoletti [69]. *Roussioella* is characterized by immersed clypeate ascostromata, pseudoparaphyses embedded in a gelatinous matrix, bitunicate asci, and ornamented, two-celled, brown, longitudinally striate ascospores [70,71]. Species of *Roussioella* can be found on bamboo and woody plants and in seawater [49,71–74]. Currently, 39 species of *Roussioella* are listed in Index Fungorum (2021). The asexual morph of *Roussioella doimaesalongensis* was found only on the host substrates in nature [49]. Attempts to stimulate sporulation of our strain isolated from freshwater were unsuccessful. Many nutritional factors are effective in fungal sporulation such as sources of carbon and nitrogen, as well as microelements [75,76]. Some fungi have specific carbon and nitrogen requirements for sporulation [76–78]. Thus, in the course of studying this species, we attempted to

use different culture media and varying conditions to induce sporulation; however, all attempts to induce sporulation failed. Poli et al. [74] have previously reported that *Roussioella* spp. isolated from seawater failed to sporulate.

The knowledge of taxonomy, distribution, and ecology of Dothideomycetes and Sordariomycetes is still poor in Korea. More works are needed to better understand the diversity and ecology of these taxa.

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No potential conflict of interest is reported by the author(s).

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